

NOVEL STABLE POLYMORPHIC FORMS OF AN ANTICONVULSANT

The present invention relates to novel stable polymorphic forms of an anticonvulsant, tiagabine hydrochloride (INN name) used in the treatment of epilepsy.

BACKGROUND OF THE INVENTION:

United States Patent No. 5,010,090 (assigned to Novo Nordisk, referred to hereinafter as '090) discloses tiagabine hydrochloride and the process of its preparation. The process adopted herein is very laborious and expensive as it utilizes column chromatography for purification. Further, the product is crystallised using ethyl acetate, isopropanol, acetone or water yielding product contaminated with high levels of solvent. Use of alternative organic solvents such as acetonitrile, butylacetate, toluene, acetone, dichloromethane etc. also gives product containing various amounts of the used crystallization solvent. The crystallization solvents are unwanted as they affect the stability of pharmaceutical products and are toxic to humans. Further the product manufactured using ethylacetate and other organic solvents often forms clathrates, hence not usable as pharmaceutical material due to high levels of solvent contamination. This patent does not disclose the polymorphic form of tiagabine hydrochloride.

United States Patent No. 5,354,760 (assigned to Novo Nordisk, referred to hereinafter as '760) patent provides monohydrate form of tiagabine hydrochloride referred to herein as form I. The monohydrate form of tiagabine hydrochloride is stable, non-hygroscopic and is suitable for pharmaceutical formulations as the only residual solvent in the product is water. However, it is reported that the monohydrate crystalline form is less stable at elevated temperature making its use inconvenient during formulation. The '760 patent discloses a process of preparation of form I tiagabine hydrochloride (monohydrate) comprising crystallization of tiagabine hydrochloride from an aqueous solution.

United States Patent No. 5,958,951 (assigned to Novo Nordisk, referred to hereinafter as '951) claims anhydrous crystalline form of tiagabine hydrochloride referred to herein as form II. The product obtained was reported to be non-hygroscopic and thermally stable. The process for preparation of form II claimed in '951 was the same as the process disclosed in '760, however the examples differ with respect to the conditions of crystallization for example in the exemplified process of '951 the crystallization from aqueous solution may occur at high temperature of about 52°C over a period of about 18 hours. Thus, the process for the preparation of anhydrous form is time consuming.

OBJECT OF THE INVENTION:

The object of the present invention is to provide novel stable polymorphic forms III, IV and substantially amorphous form of tiagabine hydrochloride

Another object is to provide novel solvate of tiagabine hydrochloride with acetonitrile.

Yet another object of the present invention is to provide processes for the preparation of novel polymorphic forms III, IV, novel solvate with acetonitrile and substantially amorphous form of tiagabine hydrochloride.

SUMMARY OF THE INVENTION

The present invention provides novel stable polymorphic forms of tiagabine hydrochloride, an anticonvulsant

Particularly, the present invention provides novel stable polymorphic forms III and IV of tiagabine hydrochloride.

More particularly, the present invention provides stable polymorph IV of tiagabine hydrochloride that exhibits an X-ray powder diffraction pattern having characteristic peaks expressed in degrees 2 theta at about 13.6, 14.5, 15.4, 16.2, 16.8, 23.0, 24.7, 26.0

The present invention also provides tiagabine hydrochloride acetonitrile solvate.

The present invention also provides amorphous tiagabine hydrochloride.

The present invention also provides a process for the preparation of each of the polymorphic forms III and IV of tiagabine hydrochloride and amorphous tiagabine hydrochloride.

DETAILED DESCRIPTION OF THE INVENTION:

When we followed the claimed process of the '951 patent i.e. by dissolving tiagabine hydrochloride in an aqueous hydrochloric acid solution, and precipitating tiagabine hydrochloride from the aqueous hydrochloric acid solution we obtained the anhydrous form II and on analysis found that the cell parameters of the form II were :

$$\begin{array}{ll} a = 7.775(7)\text{\AA} & \alpha = 78.38(9)^\circ \\ b = 11.10(1)\text{\AA} & \beta = 75.88(8)^\circ \\ c = 14.33(2)\text{\AA} & \gamma = 89.21(9)^\circ \end{array}$$

$$\text{Vol} = 1173.96 \text{ \AA}^3$$

We studied the solubility of tiagabine hydrochloride in various organic solvents and found that tiagabine hydrochloride has limited solubility in various organic solvents compared to that in water. The solubility data is given in Table-I.

**TABLE-1 : SOLUBILITY DATA OF TIAGABINE HYDROCHLORIDE AT
ROOM TEMPERATURE**

S.NO	SAMPLE QUANTITY	SOLVENT	VOLUME OF SOLVENT
1	100 mg	Toluene	>100ml
2	100 mg	DMF	0.5 ml
3	100 mg	Ethylacetate	>100 ml
4	100 mg	Acetone	24 ml
5	100 mg	Methanol	0.2 ml
6	100 mg	Ethanol	0.3 ml
7	100 mg	IPA	1.3 ml
8	100 mg	Acetonitrile	>100 ml
9	100 mg	Water	0.7 ml

Further, whereas the prior art method of crystallization from aqueous solution was time consuming we found that process using crystallization from organic solvents was rapid and resulted in high yields.

The stable polymorphic form III of tiagabine hydrochloride exhibits an x-ray diffraction pattern as depicted in fig. 1.

The stable polymorphic form IV of tiagabine hydrochloride exhibits an x-ray diffraction pattern as depicted in fig. 2.

According to one embodiment of the present invention novel stable polymorphic forms of tiagabine hydrochloride may be made available which are stable. Preferably, polymorphic forms III, IV and acetonitrile solvate of tiagabine hydrochloride.

The stable polymorph IV of tiagabine hydrochloride exhibits an X-ray powder diffraction pattern having characteristic peaks expressed in degrees 2 theta at approximately 13.6, 14.5, 15.4, 16.2, 16.8, 23.0, 24.7. Preferably, x-ray powder diffraction pattern having characteristic peaks expressed in degrees 2 theta at about 4.46, 5.03, 5.48, 6.46, 7.46, 8.11, 8.35, 9.45, 10.29, 11.41, 11.94, 12.32, 12.91, 13.59, 13.83, 14.52, 14.85, 15.36, 15.97, 16.26, 16.83, 17.85, 18.36, 18.59, 18.85, 19.25, 19.45, 20.36, 20.98, 21.59, 22.15, 22.49, 22.99, 23.67, 23.96, 24.75, 25.33, 25.62, 25.97, 26.43, 27.02, 27.48, 27.94, 28.16, 28.88, 29.63, 30.27, 30.87, 31.54, 32.11, 32.52, 32.96, 33.52, 33.89, 34.45, 35.33, 35.59, 36.02, 36.53, 36.77, 37.28, 37.75, 38.24, 39.12.

More preferably, the stable polymorph IV of tiagabine hydrochloride exhibits x-ray powder diffraction pattern as given below:

2θ	% Relative Intensity	2θ	% Relative Intensity
4.4552	1.19	32.9663	1.20
5.0280	1.57	33.5206	2.07
5.4800	1.40	33.8931	5.86
6.4618	26.01	34.4505	1.63
7.4555	1.14	35.3347	6.23
8.1120	1.05	35.5891	4.77
8.3469	0.55	36.0204	0.29
9.4458	1.10	36.5288	0.90
10.2976	0.07	36.7720	0.81
11.4113	10.74	37.2771	1.76
11.9400	0.48	37.7485	0.72
12.3205	0.54	38.2364	0.52
12.9051	73.52	39.1197	1.45
13.5866	34.36		
13.8336	22.44		
14.5237	21.87		
14.8525	56.45		
15.3566	100.00		
15.9704	19.58		
16.2550	37.61		
16.8324	58.66		
17.8470	3.19		
18.3616	8.99		
18.5960	9.64		
18.8520	23.20		
19.2462	43.35		
19.4466	35.71		
20.3582	7.12		
20.9813	10.55		
21.5955	2.40		
22.1478	7.55		
22.4936	13.01		
22.9961	37.54		
23.6666	13.88		
23.9563	22.03		
24.7460	64.16		
25.3288	14.29		
25.6240	16.37		
25.9657	16.05		
26.4322	8.81		
27.0201	8.24		
27.4772	2.36		
27.9365	4.32		
28.1570	6.49		
28.8818	3.13		
29.6343	3.57		
30.2723	3.56		
30.8721	0.87		
31.5401	1.93		
32.1129	0.31		
32.5239	2.94		

Stable polymorph IV of tiagabine hydrochloride exhibits unit cell parameters as given below:

$$\begin{aligned}a &= 10.788(3)\text{\AA} & \alpha &= 97.65(2)^\circ \\b &= 11.492(2)\text{\AA} & \beta &= 108.92(2)^\circ \\c &= 14.799(4)\text{\AA} & \gamma &= 101.86(2)^\circ\end{aligned}$$

$$\text{Vol} = 1658.63 \text{ \AA}^3$$

In embodiment of the present invention the stable polymorph IV of tiagabine hydrochloride may be obtained in particle size with volume mean diameter (VMD) less than 20 microns.

There is no change in the x-ray diffraction pattern of stable polymorph IV of tiagabine hydrochloride after standing for 6 months under ambient conditions.

The process for the preparation of the novel stable polymorphic forms III or IV of tiagabine hydrochloride of the present invention comprises dissolving tiagabine hydrochloride to an organic solvent or a mixture of organic solvent and organic anti-solvent and adding a sufficient amount of organic non-solvent to the solution to cause crystallization at a selected temperature wherein the selected temperature is such that form IV of tiagabine hydrochloride is crystallized. The organic solvent may be water miscible or water immiscible. Water miscible organic solvent may be used alone or in admixture with water.

In accordance with another embodiment of the present invention, there is provided a process for the preparation of novel stable polymorphic form III and form IV of tiagabine hydrochloride.

A process for the preparation of the novel stable polymorphic form IV of tiagabine hydrochloride comprises dissolving tiagabine hydrochloride in an organic solvent or a mixture of organic solvent and an organic non-solvent and adding a sufficient amount of organic anti-solvent to the solution to cause crystallization at a selected temperature wherein the selected temperature is such that form IV of tiagabine hydrochloride is crystallized preferably the selected temperature may be $35 \pm 10^\circ\text{C}$. The solution may be optionally cooled at 0° to 10°C for further crystallization.

The novel stable polymorphic form IV of tiagabine hydrochloride may also be prepared by crystallizing crystallizing tiagabine hydrochloride from a solution of tiagabine hydrochloride in an organic solvent or a mixture of organic solvent and organic anti-solvent wherein the solution is seeded with tiagabine hydrochloride form IV seed crystals.

A process for the preparation the novel stable polymorphic form III of tiagabine hydrochloride comprises adding tiagabine hydrochloride in an organic solvent, heating to dissolve and adding sufficient amount of organic anti-solvent to cause crystallization at a selected temperature wherein the selected temperature is such that form III of tiagabine hydrochloride is crystallized.

The novel stable polymorphic form III of tiagabine hydrochloride may also be prepared by crystallizing tiagabine hydrochloride from a solution of tiagabine hydrochloride in an organic solvent or a mixture of

organic solvent and organic anti-solvent wherein the solution is seeded with tiagabine hydrochloride form III seed crystals.

The organic solvent may be selected from the group consisting of aliphatic or aromatic or cyclic hydrocarbon such as n-pentane, n-hexane, n-octane, cyclohexane, toluene and the like; halogenated aliphatic or aromatic hydrocarbons such as dichloromethane, chlorobenzene; alkanols such as methanol, ethanol, t-butanol, isopropanol, cyclohexanol and the like; ethers such as diethylether, tetrahydrofuran, dioxane; ketones such as acetone, methylethylketone, cyclohexanone; nitriles such as acetonitrile; amides such as dimethylformamide, dimethylacetamide and the like; esters such as ethylacetate, butylacetate ; sulfoxides such as dimethylsulfoxide and the like; water and mixtures thereof.

The preferred organic solvents used are polar aprotic organic solvents such as dimethylformamide or dimethylsulfoxide. The preferred organic anti-solvent is toluene.

The dissolution of tiagabine hydrochloride in solvent(s) may be carried out at ambient or at elevated temperatures.

In a preferred embodiment of the invention, the novel stable polymorphic form IV of tiagabine hydrochloride is prepared by dissolving tiagabine hydrochloride in a mixture of dimethylformamide and toluene, followed by adding sufficient quantity of toluene to the resulting solution at room temperature.

In another preferred embodiment of the invention, the novel stable polymorphic form III of tiagabine hydrochloride is prepared by adding tiagabine hydrochloride to dimethylformamide, heating to dissolve and adding sufficient amount of toluene to cause crystallization at a temperature ranging from 50 to 55°C.

Isolation of the novel polymorphic forms III or IV may be achieved by using techniques such as filtration/centrifugation and drying. Filtration may be carried out in the presence or absence of vacuum. Drying may be carried out at ambient or elevated temperature in the presence or absence of vacuum. The product may be dried using different techniques such as fluid bed drying, tray drying, spray freeze drying and rotatory drying techniques with or without application of vacuum and / or under inert conditions.

The new polymorphic forms III and IV of tiagabine hydrochloride are suitable for pharmaceutical formulations.

The organic solvent may be selected from the group consisting of aliphatic or aromatic or cyclic hydrocarbon such as n-pentane, n-hexane, n-octane, cyclohexane, toluene and the like; halogenated aliphatic or aromatic hydrocarbons such as dichloromethane, chlorobenzene; alkanols such as methanol, ethanol, t-butanol, isopropanol, cyclohexanol and the like; ethers such as diethylether, tetrahydrofuran, dioxane; ketones such as acetone, methylethylketone, cyclohexanone; nitriles such as acetonitrile; amides such as dimethylformamide, dimethylacetamide and the like; esters such as ethylacetate, butylacetate ; sulfoxides such as dimethylsulfoxide and the like; water and mixtures thereof.

The dissolution of tiagabine hydrochloride in solvent(s) may be carried out at ambient or at elevated temperatures.

Crystallization of tiagabine hydrochloride from the solution may be carried out at ambient or lower temperatures. Crystallization may be allowed to occur by chilling or seeding or scratching the glass of the reaction vessel or cooling and other such common techniques.

Isolation of the novel polymorphic forms may be achieved by using techniques such as

filtration/centrifugation and drying. Filtration may be carried out in the presence or absence of vacuum.

Drying may be carried out at ambient or elevated temperature in the presence or absence of vacuum. The product may be dried using different techniques such as fluid bed drying, tray drying, spray freeze drying and rotatory drying techniques with or without application of vacuum and / or under inert conditions.

For instance, polymorphic forms III or IV of tiagabine hydrochloride may be prepared by dissolving in polar aprotic solvent such as dimethylformamide or dimethylsulfoxide and the like. The dissolution may be carried out at ambient or higher temperature

This is followed by addition of anti-solvent selected from aliphatic or aromatic hydrocarbon solvents such as hexane, heptane, cyclohexane, cycloheptane, benzene, toluene, xylene and the like to crystallize polymorphic forms III or IV within about 3 hrs at ambient or lower temperature, preferably -10 to 30°C.

The new polymorphic forms III and IV of tiagabine hydrochloride are suitable for pharmaceutical formulations.

According to yet another embodiment of the present invention of tiagabine hydrochloride acetonitrile solvate may be obtained, preferably crystalline tiagabine hydrochloride acetonitrile solvate. Crystalline form of tiagabine hydrochloride acetonitrile solvate is stable and isolable in good yields.

Crystalline form of tiagabine hydrochloride acetonitrile solvate exhibits an X-ray powder diffraction pattern having characteristic peaks expressed in degrees 2 theta at approximately 7.9, 21.5, 22.0, 24.3, 24.9, 26.7, 27.8.

Crystalline form of tiagabine hydrochloride acetonitrile solvate exhibits an x-ray diffraction pattern as depicted in fig. 3.

According to another embodiment of the present invention substantially amorphous tiagabine hydrochloride may be made available.

Amorphous tiagabine hydrochloride may be prepared by various methods such as spray drying solution comprising tiagabine hydrochloride or freeze drying solution comprising tiagabine hydrochloride and the like. XRD analysis of amorphous tiagabine hydrochloride as prepared by spray drying and freeze drying are as given in figures 4 and 5.

The process for the preparation of tiagabine hydrochloride acetonitrile solvate comprising dissolving in acetonitrile or mixture comprising acetonitrile and crystallizing by cooling or standing at ambient temperature.

We have also found that the solvates of tiagabine hydrochloride can also be employed for making the new forms for eg. stable acetonitrile solvate having 1 mole of acetonitrile when dried at 85-90°C under vacuum yields form III of tiagabine hydrochloride.

The polymorphic forms III, IV, acetonitrile solvate and amorphous form of tiagabine hydrochloride are obtained from organic solvents or from drying of the solvates and had solvent levels below the acceptable limits, meeting ICH requirements. The data was reported in Table-2.

TABLE-2 : RESIDUAL SOLVENT DATA

S.no.	Exp. No.	Form	Solvent(s) used for	Solvent content	Limits as per
1.	630/12	IV	DMF +	Not detected	NMT 880 ppm
2	630/16	III	DMF +	Not detected	NMT 880 ppm
2.	630/37 _a	I	ETHYLACETATE	Not detected	NMT 5000
3.	630/37 _b	IV	ISOPROPANOL	10 ppm	NMT 5000
4.	630/37 _c	IV	ACETONE	556 ppm	NMT 5000
5.	616/20B	IV	METHANOL+	Not detected	NMT 3000
6	641/04a	Acetonitrile	ACETONITRILE	Not detected	NMT 410 ppm

Stable polymorphic forms III, IV and amorphous forms are substantially free of solvent.

The invention is further illustrated but not restricted by the description in the following examples.

EXAMPLES

Example 1 : Form -III of tiagabine hydrochloride

66 gm of tiagabine hydrochloride is dissolved in 135 ml DMF at 60-70°C and the solution filtered. 1200 ml toluene is added to DMF solution containing tiagabine hydrochloride at 50-55°C for a period of 15 min and the mixture is gradually cooled to room temperature in 1 hr period and further cooled to 0-5°C and maintained at 0-5°C for 1.5 hr. The material is filtered and washed with 150 ml toluene. Dried the material at 50-55 °C till LOD comes to less than 0.5%.

X-ray powder diffraction pattern having characteristic peaks expressed in degrees 2 theta at 6.4617, 9.3296, 11.3101, 12.9202, 13.7893, 14.4799, 14.9003, 15.3375, 15.9390, 16.2009, 16.5963, 16.7774, 18.5875, 19.4396, 20.3969, 22.4225, 23.0653, 23.6163, 23.9868, 24.6971, 25.2271, 25.9469, 26.4799, 27.0214, 27.2297, 28.8106, 29.6048, 31.4648, 32.4574, 33.5262, 33.8443, 35.6166, 36.6730.

Example 2 (a) : Form-IV of tiagabine hydrochloride

650 gm of tiagabine hydrochloride is dissolved in 1.5 lit DMF at 70-80°C, and added to 6.5 lit toluene at room temperature for a period of 30 min and the mixture is gradually cooled to room temperature in 30 min time and further cooled to 5-10°C in 30 min time and maintained at 5-10°C for 2 hrs. The material is filtered and washed with 1.3 lit toluene. Dried the material at 55-58 °C till LOD comes to less than 0.5%.

(LOD Result 0.1%).

XRD analysis of form IV after 6 months storage at ambient conditions, matches the XRD of '0' day sample.

X-ray powder diffraction pattern of this product exhibited characteristic peaks expressed in degrees 2 theta 4.1162, 4.9336, 6.4616, 6.9249, 8.0731, 9.3211, 10.3290, 11.3203, 11.5021, 12.3275, 12.8948, 13.5577, 13.8687, 14.5032, 14.7572, 14.9428, 15.3449, 15.9370, 16.2314, 16.3377, 16.6598, 16.8500, 17.3261, 17.3037, 17.8229, 18.3380, 18.6349, 18.8832, 19.1816, 19.4174, 19.8286, 20.3221, 20.9559, 21.2182, 21.6159, 22.1136, 22.4293, 22.9777, 23.6307, 23.9568, 24.3669, 24.7233, 24.9424, 25.2110, 25.5718, 25.9348, 26.1401, 26.5171, 26.8243, 27.0467, 27.5428, 27.9526, 28.1313, 28.6444, 28.8638, 29.5891, 29.9740, 30.3277, 30.7402, 31.4942, 32.0050, 32.4651, 32.9536, 33.5620, 33.9135, 34.4093, 35.2921, 365.7069, 36.8032, 37.2098, 37.8744, 39.0016, 39.2218, 39.6847.

Particle size analysis (analysed by HELOS (H1551) & RODOS)

Form IV of tiagabine hydrochloride exhibited VMD = 16.7 and 19.8 microns for 2 batches..

Form I of tiagabine hydrochloride exhibited VMD = 60.2 microns.

Example 2 (b) : Form-IV of tiagabine hydrochloride

Charge filtered 2 parts by volume (w.r.t weight of crude tiagabine hydrochloride) of Dimethylformamide in to the RBF between 28°C ~ 32°C. Charge filtered 2 parts by volume (w.r.t weight of crude tiagabine hydrochloride) of Toluene in to the RBF between 28°C ~ 32°C. Start stirring & charge crude tiagabine hydrochloride into the RBF between 28°C ~ 32°C. Stir the content for 10 min. between 28°C ~ 32°C in the RBF to get uniform slurry. Heat the content to 65°C ~ 70°C into RBF to get a clear solution.

Charge filtered 18 parts by volume of toluene (w.r.t weight of crude tiagabine hydrochloride) into reaction mixture between 65°C ~ 70°C under stirring. Gradually cool the content between 28°C ~ 32°C. Stir the content for 45 ~ 60 min. between 28°C ~ 32°C in the RBF. Further cool the content between 0°C ~ 5°C. Stir the content for 40 to 60 min. between 0°C ~ 5°C in the RBF. Filter the product between 0°C~5°C through centrifuge. Spin dry product for 30 mins. Wash the cake twice with chilled toluene. Spin dry the product for 60 mins.

X-ray powder diffraction pattern of this product exhibited characteristic peaks expressed in degrees 2 theta at 4.4552, 5.0280, 5.4800, 6.4618, 7.4555, 8.1120, 8.3469, 9.4458, 10.2976, 11.4113, 11.9400, 12.3205, 12.9051, 13.5866, 13.8336, 14.5237, 14.8525, 15.3566, 15.9704, 16.2550, 16.8324, 17.8470, 18.3616, 18.5960, 18.8520, 19.2462, 19.4466, 20.3582, 20.9813, 21.5955, 22.1478, 22.4936, 22.9961, 23.6666, 23.9563, 24.7460, 25.3288, 25.6240, 25.9657, 26.4322, 27.0201, 27.4772, 27.9365, 28.1570, 28.8818, 29.6343, 30.2723, 30.8721, 31.5401, 32.1129, 32.5239, 32.9663, 33.5206, 33.8931, 34.4505, 35.3347, 35.5891, 36.0204, 36.5288, 36.7720, 37.2771, 37.7485, 38.2364, 39.1197

Example 3 : Amorphous form of tiagabine hydrochloride

25 gm tiagabine hydrochloride is dissolved in 125 ml methanol + water mixture in 1:1 ratio at room temperature and spray dried the material at 45-50°C . It can also be prepared by dissolving 25 gm tiagabine hydrochloride in 175 ml water at 50-55°C temperature and spray dried the material at 60°C. Another method of preparing amorphous form is by dissolving 10 gm tiagabine hydrochloride in 110 ml distilled water at room temperature and freeze dried the material for 24 hrs. XRD analysis are given in Fig. 4.

Example 4 : Monoacetonitrile solvate of tiagabine hydrochloride

5 gm of tiagabine hydrochloride is dissolved in 5 ml of methanol at 50-55°C, 50 ml acetonitrile is added to the methanol solution at 40-55°C and cooled to room temperature in 1 hr period and further cooled to 5-10°C and stirred for 2 hrs. Allowed the product to settle down and decanted the clear liquid. 50 ml ethyl acetate is added to the solid mass and stirred at 5-10°C for 30 min, allowed the product to settle down the and decanted the clear liquid. Once again 50 ml ethyl acetate is added to the solid mass and stirred at 5-10°C for 30 min, allowed the product to settle down the and decanted the clear liquid and dried the product mass in rotavapour under mild vacuum at 50°C for 2hrs.

The obtained acetonitrile solvate form was dried at 85-90°C under vacuum to obtain form III of tiagabine hydrochloride.

X-ray powder diffraction pattern having characteristic peaks expressed in degrees 2 theta at 7.8620, 11.7636, 12.7349, 13.4762, 14.3981, 14.8732, 15.7568, 16.8937, 17.1116, 17.4938, 18.0955, 18.8451, 19.8842, 21.5213, 22.0078, 23.2299, 23.6888, 24.2776, 24.6823, 24.9106, 25.6034, 26.2117, 26.6924, 27.5132, 27.7983, 28.4213, 28.9876, 29.7388, 30.1996, 30.5997, 31.5065, 31.5065, 32.7371, 36.1356, 38.1619.